REPORT

OF THE

PLAGUE RESEARCH LABORATORY

FOR THE

Official Year ending 31st March 1905.

BY

LIEUT.-COLONEL W. B. BANNERMAN,
M.D., B.Sc., F.R.S.E., I.M.S.,
DIRECTOR OF THE LABORATORY.

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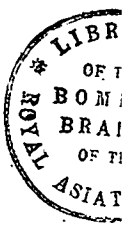

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REPORT OF THE PLAGUE RESEARCH LABORATORY FOR THE OFFICIAL YEAR ENDING 31ST MARCH 1905.

In the year under report the post of Director was held by Mr. W. M. Haffkine, C.I.E., from 1st to 30th April; by Dr. F. Maitland Gibson from 30th April to 5th May, and by the writer for the rest of the year.

The staff consisted of F. Maitland Gibson, M.B., B.Sc. (Edin.), and Captain W. Glen Liston, M.D. (Glasgow), I.M.S. (who rejoined on 9th May 1904), as Assistants to the Director; and E. S. Winter,* M.R.C.S. (Eng.), L.R.C.P. (London), Inspector of Inoculation. For varying periods W. G. West,* M.R.C.S. (Eng.), L.R.C.P. (London), M.D. (Geneva), and R. D. Dalal,* M.R.C.S. (Eng.), L.R.C.P. (London), were employed on the superintendence of the trial of Roux' serum in the Maratha Plague Hospital, and in collecting statistics from various parts of India in regard to the effect on human communities of the various sorts of plague prophylactic employed during the previous year. Senior Assistant Surgeon R. J. Kapadia was as before in charge of the testing of the vaccine as to purity; Assistant Surgeon J. P. Pocha continued in charge of the sterilization of the vaccine, and Dr. Pansare was supervisor of decanters as in previous years.

2. In the 12 months under review, *i.e.*, from 1st April 1904 to 31st March 1905, 129,507 doses of plague prophylactic vaccine were manufactured and issued from the Laboratory.

In India 122,683 doses were sent to the following provinces:—

British India.		
Bombay Presidency	58,839	
United Provinces of Agra and Oudh	7,374	
Berar	5,942	
Madras Presidency	4,029	
Bihar	3,888	
Central Provinces	2,014	
The Punjab	1,304	
Burma	1,279	
Bengal	605	
Assam	250	
Total for British India ...		85,524
Native States.		
Central India	26,544	
Nizam's Government	5,496	
Mysore Government	2,006	
Rajputana	1,851	
Bombay Presidency	1,238	
Malwa Agency	24	
Total for Native States ...		37,159
Total for India ...		122,683

* Medical men engaged under the orders of the Secretary of State for India.

Outside India.

Ceylon	...	2,000
Persian Gulf	...	1,809
China	...	1,802
Straits Settlements	...	711
Siam	...	502
Total for Countries outside India	...	6,824
Grand Total	...	129,507

The total number of doses of plague prophylactic vaccine issued from the Laboratory since 10th January 1897 when Mr. Haffkine had the first inoculation performed on himself, amounts now to 5,886,732 doses. Of these 5,374,942 doses were issued to operators in India and 511,790 to places outside this country.

Since the occurrence of the Malkowal disaster in November 1902, a gradual diminution in the demands has taken place as may be seen from the comparison of the amounts issued month by month for the past two years :—

April	1903	...	7,646	April	1904	...	3,647
May	„	...	16,577	May	„	...	2,536
June	„	...	2,896	June	„	...	2,844
July	„	...	5,682	July	„	...	4,950
August	„	...	11,820	August	„	...	19.288
September	„	...	20,787	September	„	...	12,963
October	„	...	32,767	October	„	...	5,012
November	„	...	18,358	November	„	...	15,864
December	„	...	23,472	December	„	...	6.281
January	1904.	...	14,050	January	1905	...	41,755
February	„	...	12,062	February	„	...	7,949
March	„	...	16,252	March	„	...	8,579
	Total	...	182,369		Total	...	131,668

As the publication of the Government communiqué on the cause of this disaster (*vide* Appendix A) becomes known and people realise that there is now no chance of such another accident taking place, it is to be hoped that inoculation will take its place once more as the important measure it undoubtedly is.

It cannot be too often, or too emphatically, stated that the cause of the nineteen deaths from tetanus at Malkowal was the omission of the carbolic acid from the vaccine. Had this been present no tetanus germs could have grown in the prophylactic fluid, and therefore no tetanus cases would have occurred.

It is hardly necessary to state that since the discovery by the experimenters in India, and its confirmation at the Lister Institute, that carbolic acid has this inhibiting action in the case of the plague prophylactic, it has been an invariable rule to add the 0·5 per cent. of carbolic acid.

3. During the year several changes in procedure have been made which, it is believed, have resulted in improvement in the quality of the vaccine turned out :—

(1) The enriching of the prophylactic vaccine by the addition of fresh plague germs to the fluid at the close of the incubation period has been discontinued.

(2) The germs used as seed material are now invariably as virulent as possible, and not more than two weeks elapses from the securing of the germs until they are sown in the large flasks containing the broth which ultimately constitutes the vaccine material.

(3) The method of manufacturing the bouillon is that worked out during the year by Dr. F. Maitland Gibson and detailed below. (*Vide* Appendix B.)

(4) In standardising the fluid reliance is now placed on the exact procedures employed in obtaining fresh and virulent germs and for securing a nutrient bouillon of nearly constant composition, checked by animal experiment from time to time.

(5) The method of bottling now used is that invented by Dr. E. Maynard, which obviates any chances of contamination while the fluid is passing from the culture flask to the bottle, and which provides for its subsequent safety by hermetical sealing.

These changes have been adopted after an extensive series of experiments, and the following is a brief summary of them.

(1) *The enriching of the Prophylactic.*—This process consists in adding to the 6 weeks old broth culture of plague, which constitutes the original or "Standard" prophylactic, a large number of fresh plague microbes grown, for four days only, on agar plates.

A great increase in thickness is thus produced by the addition of these fresh bacillary bodies, and to all appearance a stronger prophylactic is obtained. *A priori* one would be inclined to agree with the advocates of this process,* that the addition of fresh microbes must increase the effectiveness of the prophylactic, but judged by the results of experiments on a large number of animals, this is found not to be the case. The animals experimented on, whether they were inoculated with the reduced dose prescribed for this thickened prophylactic vaccine, or with a dose equal in amount to that of the standard prophylactic, seemed, after infection with plague, to be struggling with some restraining factor from which the animals protected by the standard vaccine were free. From experiments on guinea-pigs, rats, and two species of monkeys it was found that the standard vaccine was at least 10 times more efficient than the enriched vaccine made from the same strain of germs, when the latter was given in the proportionately smaller amount prescribed owing to its thickness.

A second series of experiments with the same brews, but where the enriched vaccine was given in the same doses as the standard vaccine, furnished similar results.

In an endeavour to ascertain the effect produced by the enriched vaccine when used in human communities, a great amount of painstaking labour has been expended by Drs. Winter, Dalal and West. These medical officers visited a large number of villages in Ahmednagar, Central India, the Central Provinces, and Berar, where inoculation work had been carried on in the previous season with one or other sorts of prophylactic, or where both had been used conjointly.

The following tables show briefly the results obtained where accurate information as to the brews used, was forthcoming. Table I is compiled from villages where every house inhabited by an inoculated individual was visited, and the results recorded, whether plague had entered the house or not; the contrasting uninoculated population being in this case not only those living in the same houses as the inoculated, but also the whole of the rest of the inhabitants of these villages. Table II represents the results obtained from a census of infected houses only, and does not include all inhabitants in the villages dealt with :—

TABLE I.

		Attacks.	Deaths.	Case incidence per cent.	Death incidence per cent.	Case mortality per cent.	Percentage immunity of inoculated from death.
Inoculated with standard vaccine ...	1,381	36	25	2·6	1·8	69·4	73·9
Not inoculated ...	14,343	1,183	1,005	8·2	7·0	84·9	...
Inoculated with enriched vaccine ...	556	18	12	3·2	2·1	66·4	63·6
Not inoculated ...	15,649	1,257	953	8·0	6·0	75·8	...

* *Vide* Indian Plague Commission's Report, Vol. III, p. 644, letter from Mr. Haffkine to Secretary to Government of India, Home Department.

TABLE II.

	Attacks.	Deaths.	Case incidence per cent.	Death incidence per cent.	Case mortality per cent.	Percentage immunity of inoculated from death.
Inoculated with standard vaccine 331	64	44	19·3	13·2	68·7	50·0
Not inoculated ... 1,177	389	314	33·05	26·6	80·7	...
Inoculated with enriched vaccine 774	222	136	28·6	17·5	61·2	35·8
Not inoculated ... 2,525	842	680	33·3	26·9	80·7	...

Looking at these figures, and comparing them with the results obtained in former years, one is struck with the lessened effect produced by both sorts of prophylactic. Formerly one looked for an immunity from deaths among the inoculated of over 80 per cent. and frequently a good deal more, taking the whole population of a village into account; but in this case an immunity of 73·9 per cent. for the standard and 63·6 per cent. for the enriched vaccine was obtained.

Taking infected house only, we find an immunity from deaths of 50 per cent. for the standard and 35·8 per cent. for the enriched prophylactic, as compared with 89·6 per cent. for similar cases where the original prophylactic was employed, such as in Undhera for instance.

It is probable that the comparatively poor results shown above may be due to the age of the germs used in the manufacture of these particular vaccines. In any case as both sorts of vaccine were equally made from old germs, any deductions drawn from the results obtained by their use are strictly comparable from this point of view. If this is admitted, then it is evident that results obtained among human communities, bear out the conclusions arrived at by experiments on animals, *viz.*, that the enriched prophylactic is not so effective as that made by the old standard method.

(2) *The virulence of the germs.*—It is reasonable to suppose that a virulent germ will produce a better vaccine than an a-virulent one.

This was found to be the case.

It was ascertained that growth on agar for 23 days had the effect of lowering the virulence of a particular germ 250 times. This growth on agar for many days, used to be followed here as a routine practice when isolating the germ in pure culture from the body prior to sowing the flasks, but since the above discovery was made, this practice has been discontinued, and one remove to broth has been substituted.

A certain number of seed stocks have no doubt to be rejected when this method is followed, but the loss of material is nothing compared to the gain in quality of the vaccine which it is believed has resulted.

Since the above was worked out, confirmation of these ideas has been furnished by an American bacteriologist in Manila,* working with the cholera spirillum, who found (page 36) "that the immunity obtained is within certain limits proportional to the virulence of the inoculated strain."

Since virulent germs were used for making the vaccine, an inoculation compaign on a large scale has been carried out at Aden.

Altogether 9,374 inoculations were performed between 18th December 1904 and April 20th, 1905.

Owing to the fact that a large part of the population of Aden was composed of homeless wanderers and famine refugees, it was impossible to verify the statistical returns for the whole population.

Several compact communities, however, were found where it was possible by house-to-house inspection to compile a correct census of the population living there when the inoculations were performed. The results are very favourable as may be gathered by reference to the report of Dr. Winter printed as Appendix D.

* Protective Inoculation against Asiatic Cholera (an experimental study), by Richard P. Strong, M.D.; No. 16, September 1904. Manila Bureau of Public Printing, 1904.

(3) *The manufacture of the bouillon.*—The improvement effected in the manufacture of the bouillon seems to be a very real one, both on the grounds of economy and efficiency.

Formerly the goat's flesh forming the basis of the broth was kept in a water bath at 70° Cent. for 7 days, and was then heated for 2 hours under a pressure of 3 atmospheres.

Now it is kept in the water bath for three days only, and then heated for one hour at 100° Cent. In this way a broth of much lighter colour is obtained, and one in which the plague bacillus seems to grow more freely. It is hoped, on this latter account, that it may be possible eventually to reduce the bulk of the dose of prophylactic which is needed to afford adequate protection to human beings. The details of the manufacture are set forth in Appendix B by Dr. F. Maitland Gibson, who worked out the method.

(4) *Standardising the vaccine.*—The difficulty hitherto met with when trying to standardize a vaccine, has been the impossibility of ascertaining the minimum lethal dose (M. L. D.) for the living germs employed.

For, it is manifest, that if the quantity of poison sufficient to kill an experimental animal cannot be ascertained, neither can the amount of antidote be measured which will suffice to counteract it.

By a modification of Wright's ingenious method of actually counting the number of germs in a given culture, Captain Liston has overcome this difficulty, and paved the way for a solution of the problem of accurate standardization of vaccines, for those diseases which can be communicated to animals, as plague can. He found it possible by the technique described in the Appendix C to estimate the actual number of separate entities of any particular germ necessary for the killing of the species of animals worked with. Having ascertained this, it became possible to try one vaccine against another, and, in this way, to find out which was the best. As the result of many trials, it was determined that, pending the result of experiments on the production of a "negative phase" or period of increased susceptibility following inoculation, the following should be fixed as a suitable standard for the plague vaccine, *viz.*: "The maximum quantity of a vaccine which will, in 48 hours, protect a guinea-pig weighing 250 to 300 grammes, against at least 100 times the lethal dose of a plague culture isolated from the body of an animal not more than one week previously."

It was proposed to call this "one unit" and to recommend its use in the case of persons *living in infected households*, the dose for all others to be two units or 5 c.c.

Since then two series of experiments have been carried out, with results which seem to indicate that no negative phase is produced by injection of the plague vaccine.

The following is a summary of these experiments :—

First Experiment.

Group I.—Simultaneously vaccinated and inoculated.

Controls.	2·5 c.c. vaccine.	5 c.c. vaccine.
Mean number of days of life of 4 Guinea-pigs. 7 days.	Mean number of days of life of 4 Guinea-pigs; with same doses as controls. 10 days.	Mean number of days of life of 4 Guinea-pigs; same doses as controls. 7 days.

Group II.—Inoculated 12 hours after vaccination.

Controls.	2·5 c.c. vaccine.	5 c.c. vaccine.
Mean number of days of life of 3 Guinea-pigs. 8 days.	Mean number of days of life of 3 Guinea-pigs; with same doses as controls. 11 days.	Mean number of days of life of 3 Guinea-pigs; with same doses as controls. 10 days.

Group III.—Inoculated 24 hours after vaccination.

Controls.	2·5 c.c. vaccine.	5 c.c. vaccine.
Mean number of days of life of 3 Guinea-pigs. 13 days.	Mean number of days of life of 5 Guinea-pigs, 3 with same doses as controls, 2 with 10- and 100-fold the dose given to controls. 11 days. *N. B.*—One of the 5 Guinea-pigs recovered.	Mean number of days of life of 5 Guinea-pigs, 3 with same doses as controls, 2 with 10- and 100-fold the dose of control. 13 days. *N.B.*—Two of the 5 Guinea-pigs recovered.

Group IV.—Inoculated 48 hours after vaccination.

Controls.	2·5 c.c. vaccine.	5 c.c. vaccine.
Mean number of days of life of 3 Guinea-pigs. 8 days.	Mean number of days of life of 5 Guinea-pigs; 2 with same doses as controls and 3 with 10-, 100-, and 1,000-times the doses given to controls. 12 days. *N.B.*—Two recovered.	Mean number of days of life of 4 Guinea-pigs, only one with same dose as control; 3 with 10-, 100-, and 1,000-times the dose given to controls. 8 days.

Group V.—Inoculated 72 hours after vaccination.

Controls.	2·5 c.c. vaccine.	5 c.c. vaccine.
Mean number of days of life of 3 Guinea-pigs. 6 days.	Mean number of days of life of 5 Guinea-pigs; 2 with same doses as controls and 3 with 10-, 100-, and 1,000-times the doses given to largest dose control. 14 days. *N.B.*—Two recovered.	Mean number of days of life of 5 Guinea-pigs; 2 with same doses as controls and 3 with 10-, 100-, and 1,000-times the dose given to largest dose control. 14 days. *N.B.*—Two recovered.

Second Experiment.

Group I.—Inoculated 24 hours after vaccination.

Controls.	2½ c.c.	20 c.c.
	Miscarried	

Group II.—Inoculated 72 hours after vaccination.

Controls.	2½ c.c.	20 c.c.
Mean of 2 6 days.	Mean of 2; with same doses as controls. 10 days.	Mean of 2; with same doses as controls. 10 days.

Group III.—Inoculated 5 days after vaccination.

Controls.	2½ c.c.	20 c.c.
Died in 7 days	Recovered	Lived 12 days.

Group IV.—Inoculated 7 days after vaccination.

Controls.	2½ c.c.	20 c.c.
Died in 8 days	Recovered	Lived 13 days.

Group V.—Inoculated 12 days after vaccination.

Controls.	2½ c.c.	20 c.c.
Died in 10 days	Died in 9 days	Lived 14 days.

From the above experiments it is evident that even with such large doses as 20 c.c. of prophylactic, the guinea-pigs were not adversely affected when subsequently infected with plague; in other words, there is no evidence of the production of a "negative phase".

This is in complete accord with the results arrived at by Major Leishman* from the examination of the blood of men treated with Wright's anti-typhoid vaccine. Regarding the question of the development of a negative phase in the case of these men, he reports (page 28): "No evidence of the development of such a phase was found in any of the groups, either on first inoculation or re-inoculation." And again he says (page 29): "It seems probable, therefore, that, with dosage such as we employed, a negative phase, if developed, is so slight or so transient in nature as to be negligible."

In view of the animal experiments performed here with plague, and the blood examinations carried out by Major Leishman and his colleagues in the case of men inoculated with anti-typhoid vaccine, it has now been resolved to fix the minimum dose of anti-plague vaccine at "two units," *i.e.*, an average of 5 c.c. for a dose.

This takes for granted that there is, in the case of plague, no appreciable negative phase to be afraid of, and this conclusion is confirmed by the result of a careful examination of results obtained among human communities where plague was epidemic at the time of inoculation. In a record of over 6,000 attacks of plague, it was found that the case-mortality in the inoculated was 43·5 per cent., while among the uninoculated it was 73·3 per cent. Amongst 358 persons attacked by plague within 10 days of inoculation the case-mortality was 48·04 per cent. Amongst 43 persons who actually had symptoms of plague at the time of inoculation, or who developed such symptoms within 24 hours, the case-mortality was 48·8 per cent.; practically the same as the average for those attacked within ten days of inoculation.†

There being, therefore, no evidence of the existence of a negative phase in plague inoculation, it is safe to administer the full dose of two units even to those living in infected houses.

Other methods of standardising which have been tested and found wanting are the following:—(*a*) The febrile reaction produced; (*b*) the opacity test; (*c*) the amount of alkali present.

The following table taken from Captain Liston's report shows this clearly. The vaccines are arranged (column 2) in order of efficiency as found after experiments on animals, and the other columns show how this agrees or disagrees with the order as judged by the various tests noted over each:—

*Anti-typhoid Inoculation Committee. Report on the blood changes following Typhoid Inoculation, by Major W. B. Leishman, R.A.M.C., Professor of Pathology, R. A. M. College. London, Harrison and Sons, 1905.

† Inoculation in the Incubation Stage of Plague, by Major W. B. Bannerman, Superintendent, Plague Research Laboratory. British Medical Journal, 1901, Vol. II, p. 669.

Number of weeks germ was allowed to grow before sterilisation.	Efficiency as judged by			
	Animal experiment.	Alkali produced.	Opacity test.	Febrile reaction.
8	1st	7th	4th	8th
6	2nd	1st	3rd	6th
5	3rd	4th	6th	5th
7	4th	3rd	5th	7th
3	5th	6th	2nd	1st
4	6th	5th	1st	4th
9*	7th	2nd	7th	2nd
2	8th	8th	9th	3rd
1	9th	9th	8th	9th

* Manufactured from an a-virulent germ.

An examination of the first two columns of the above table shows that the efficiency as proved by animal experiment corresponds fairly closely with the time the germs were grown in bouillon before the vaccine was made, for we may exclude the vaccine grown for nine weeks, as it was purposely made from an a-virulent germ for experimental purposes, and is not therefore comparable with the other vaccines. The seven-weeks vaccine is probably placed too low in the scale, as in a subsequent series of experiments when tested against the six-weeks vaccine it proved slightly more efficient.

A comparison with the other columns shows results so irregular as to contra-indicate the use of any of them as a means of standardising the vaccine.

(5) *The method of bottling the prophylactic.*—The transference of the sterilised and carbolised vaccine from the flask in which it is made, to the bottles in which it is issued, has hitherto been accomplished by syphoning into special glass bottles furnished with India-rubber stoppers. Though both bottles and stoppers were sterilised, yet the actual filling of necessity involved contact with the air which cannot be sterilised. It also involved the use of separate stoppers which had to be sterilised and kept sterile till placed in position, and the risk inseparable from these manipulations.

Dr. E. Maynard, who was one of the Laboratory staff during the stress of the Punjab scheme, realised these drawbacks, and elaborated a plan by which exposure to unsterilised air is avoided, and the use of stoppers done away with. A full account of this apparatus cannot at present be published as the patent rights are not yet secured for the inventor, but the principle on which it is worked may be briefly set forth.

The fluid to be decanted is conducted by means of a syphon to a bulbous chamber closed at the outlet by a rubber disc.

The special bottle intended to contain the vaccine or other fluid previously vacuumised and sterilised, is provided with a long drawn-out neck which can be thrust through the rubber disc closing the bulbous chamber.

By a special contrivance in the chamber the tip of the bottle neck is broken off, and, in consequence, the fluid in the chamber is forced through the neck into the bottle which becomes filled in proportion to the amount of vacuum previously created in it. This can of course be regulated at will.

The bottle neck is now withdrawn from the chamber and at once sealed off in a bunsen flame. The results obtained with this method are admirable, for not only are risks avoided at the time of bottling, but when required for use, the easiest way of opening the bottles is found to involve the sterilising of the bottle mouth itself, thus affording an additional safeguard for the person inoculated. Again, as there is no means of closing the bottle, once it is opened, the operator is not tempted to keep the remainder of the vaccine for another day.

Since the adoption of this method of bottling, 894 brews have been decanted and tested. In two instances only, were contaminations found in the vaccine, and from a study of the circumstances connected with the testing, it is probable that the fault lay with the tester's technique, not with the bottling operations.

The use of these long-necked bottles also affords a ready means of keeping a sample from each one. After the usual test has been applied to each brew, the bottles are shaken up and a portion of fluid propelled into their necks. By the use of a small blow-pipe flame the neck with its contained sample can be separated and stored for future reference if need be.

3. *Are the common domestic animals of India susceptible to plague?*—This important question was by most authorities considered as settled in the negative as regards horses, cattle, sheep and goats, by experiments such as those of Mr. Haffkine* in 1897, when preparing an anti-plague serum on the lines laid down by Roux and Yersin. The German Plague Commission also experimented with doves, cocks, geese, pigs, sheep, goats and cows, and none of the animals died, though some showed a passing reaction, with regard to which they remark †: "In judging of these experiments it must not be forgotten that the infection was more intense than is met with in natural circumstances."

Lieutenant Walton, I. M. S., when worknig in Bombay with the Indian Plague Commission‡ tried to infect pigeons with plague by hypodermic injection, but failed.

Again in Russia, London§ experimented on the common birds of that country, such as pigeons, cocks, hens, ducks, crossbills, yellow-hammers, linnets and canaries. Not only were healthy birds used, but also those whose vitality might be supposed to be lowered by such measures as starvation, cold, etc. The conclusion arrived at by the author is that birds, either in a natural condition or when subjected to such treatment as the above, are not susceptible to plague.

It was therefore matter for surprise to find that in Hongkong, Professor J. W. Simpson¶ and Dr. W. Hunter regarded it as certain that "pigs, calves, buffaloes, sheep, hens, ducks, geese, turkeys and pigeons are, in addition to rats, susceptible to plague, and particularly so when fed with plague material." The report from which this sentence is taken was submitted to the Secretary of State for the Colonies and published by his authority. It, therefore, became necessary to see by fresh experiments, carried out as exactly as possible on the lines laid down by Professor Simpson, whether the common domestic animals of India were susceptible to plague, or not.

The plague germs used for these experiments were derived from human beings. They were grown in broth and the stalactite growth watched for. This culture was then used for the injection of rats. When the rats died, spleen smears were prepared for microscopic examination; and cultures on agar were made and used subsequently to sow broth for the stalactite test. If the spleen smear showed numerous plague-like bacilli, then the rat was used for feeding the experimental animals in the following way.

The head, bladder and intestines were removed, and the whole of the rest of the body and organs cut into small pieces. This was then mixed with the usual feed of bran which the animals were accustomed to eat. The bran had been previously boiled and cooled down, before the rat flesh was mixed with it. None of the animals refused to eat this bran mash, and many of them ate greedily, picking out the rat flesh with manifest gusto.

There is no doubt therefore that the experimental animals swallowed myriads of virulent live plague germs, and had they been capable of acquiring plague by the alimentary canal, there appears to be no reason why they should not have done so in every case.

Not only was an endeavour made to infect the animals in this way, but fresh series of animals were injected hypodermically with emulsions of virulent

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* Report of the Indian Plague Commission, Vol. I, p. 14, para. 133.

† Centralblatt für Bak. 1897, pp. 453-473, Summary of the Report.

‡ Report of the Indian Plague Commission, Vol. III, p. 337, paras. 26, 315.

§ Les Oiseaux sont-ils sensible à la peste bubonique?—Par M. E. S. London. Archives des Sciences Biologique, Publices par l'Institute Imperial de Med. Expr. à St. Petersbourg. Tome VI, No. 1, p. 67.

¶ Report on the Causes and Continuance of Plague in Hongkong and Suggestions as to Remedial Measures—By J. W. Simpson, M.D., F.R.C.S., London. Waterlow and Sons, Ltd., Printers, London Wall, 1903.

plague germs derived from the organs of rats infected as described above, and the identity of which was similarly established. Again, fresh animals were scarified extensively and into these abraded spots, spleens or livers of rats as above were vigorously rubbed.

The animals used were 4 pigs, 2 calves, 4 fowls, 4 turkeys, 4 geese, 4 ducks.

In all cases the results were entirely negative, so it was not considered necessary to extend the series further, as the results obtained previously in this and other laboratories in India have been identical.

Since the above experiments were completed the Laboratory has received from Mr. Ernest Hill, Health Officer for the Colony, a "Report* on the Plague in Natal, 1902-1903," in Part II of which Mr. H. Watkins-Pitchford, F.R.C.V.S., Government Bacteriologist, details trials he had made to test the domestic animals of South Africa in accordance with Professor Simpson's plan. He says, repeated endeavours have been made to induce a fatal form of the disease by the ingestion of infected material, and by the inoculation of virulent cultures and blood, as well as by close contact with an animal in a highly infectious condition.

Of 20 hens used none became ill in any way; of 10 pigs both English and Kaffir, none died and only "one showed a transient illness due to manipulation" not to plague; of two calves neither was affected."

The conclusion appears to be warranted then, that as regards India and Natal at least, the common domestic animals need not be regarded as connected in any way with the spread of plague, being insusceptible to it.

4. *Examination of animals supposed to have died of plague.*—Twenty-three rats were received for examination; 16 of these contained plague bacilli.

Besides these rats, there were received from the Victoria Gardens, 1 Sykes' monkey, 3 Wallabies, 2 Rabbits and 2 Guinea-pigs which had died of plague.

That the disease from which these animals died was really plague was proved in the case of the Wallabies and Guinea-pigs by Captain Liston, who turned loose some fresh Guinea-pigs in the Wallabies enclosure, and found that they all became infested with fleas and ultimately died of plague. An interesting link in the chain of evidence which points to the possibility of the flea being a transmitter of plague infection.

5. *Curative Serum for Plague.*—As noted in last year's Report, page 20, a supply of curative serum was sent to Mr. Haffkine by Dr. Roux of the Pasteur Institute, Paris. The trial was made by Dr. W. G. West in the Maratha Municipal Plague Hospital, where every facility was given him through the kindness of Dr. N. H. Choksy, the Medical Officer in charge of the hospital.

A full report on the use of Dr. Roux's serum, along with reports on the use of other Sera, will shortly be published in the New Series of Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India.

The following summarises the results obtained.

The patients have been arranged in three groups according as they were treated:

(*a*) By subcutaneous injection only;

(*b*) By subcutaneous injection to begin with, followed by intravenous injection; and

(*c*) By primary intravenous injection, followed by similar injections; or, in some few cases, by subcutaneous medication.

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*Report on the Plague in Natal, 1902-1903, by Ernest Hill, M.R.C.S., L.R.C.P., D.P.H. (Cantab.), Health Officer for the Colony. Printed by Cassol & Company, Limited, London, Paris, New York, and Melbourne, 1904.

	Serum Cases.			Control Cases.		
	Number.	Deaths.	Case mortality per cent.	Number.	Deaths.	Case mortality per cent.
Patients treated according to (*a*)	43	29	67·4	43	28	65·1
Do. do. (*b*)	12	8	66·6	12	6	50·0
Do. do. (*c*)	13	8	61·5	13	7	53·8
Total ...	58	45	66·2	68	41	60·3

From a study of the case mortality of the control cases corresponding to those treated with serum as above divided, it is evident that the epidemic was declining in severity as time went on. It is not therefore possible to say that the lessened case-mortality shown by the 13 cases treated according to (*c*), is due to that treatment, for a corresponding and even greater lessening of the case-mortality is exhibited by the 13 alternate control cases receiving the ordinary treatment.

We must therefore reluctantly conclude that the serum treatment, as judged by these figures, did not affect the case-mortality in the slightest.

More favourable results have been reported from the private practice of various practitioners in Bombay, and it must be remembered that the patients in an Indian hospital have usually been ill for some time before admission.

6. *Collection of Snake Venom.*—This was continued as usual throughout the year. The venom is dried and sent in sealed tubes to the Pasteur Institute of India, Kasauli, where it is used for the production of antivenene for Cobra and Daboia venoms, and for experimental investigations as to the action of various poisons.

The snakes are sent here in response to a circular from the Government of India, and the following table shows the number and description received from the various provinces during the year :—

Name of Presidency or Provinces from which received.	Species of Snakes.							
	Naja Tripudians.	Vipera Russellii.	Echis Carinata.	Bungarus Fasciatus.	Bungarus Cœruleus.	Lachesis Anamallensis.	Non-Poisonous Snakes.	Total.
Bengal	45	8	...	...	2	...	4	59
Bombay	1	1	240	...	...	...	...	242
Central Provinces	306	59	7	1	6	...	17	396
Madras	9	...	...	...	...	2	11	22
United Provinces of Agra and Oudh ...	5	...	...	...	10	...	...	15
Grand Total ...	366	68	247	1	18	2	32	734

Three persons were treated at the Laboratory for supposed cobra bite. Of these, two were almost certainly suffering from the effects of cobra poison, while the third showed no symptoms whatever. Unfortunately the snake was not seen in any of these cases.

The effect of the injections of antivenene in the two cases showing symptoms of poisoning was almost immediate and most astonishing. In the first case where the man appeared on the point of death the injection of 30 c.c. intravenously, produced immediate improvement, so that the patient, who was collapsed, was enabled to sit up and in half an hour to walk from the room with the assistance of a friend. The second case, though not so severe, had all the symptoms of commencing intoxication with cobra venom; he too rapidly recovered after intravenous injection of 15 c.c. of antivenene. The antivenene used was made at the Pasteur Institute at Kasauli.

An antivenomous serum for cases of poisoning by bites of Vipera Russellii has been produced by Captain G. Lamb,* I. M. S., of the Pasteur Institute,

* Scientific Memoirs by Officers of the Medical and Sanitary Department of the Government of India. The Specificity of Antivenomous Sera with special reference to a Serum prepared with the Venom of Daboia Russellii, by Captain G. Lamb, M.D., I. M. S. Calcutta, Office of Superintendent, Government Printing, India, 1905.

Kasauli, and a polyvalent serum found efficient for both cobra and daboia venoms is now being issued by that Laboratory.

7. *Diagnostic Work.*—A certain amount of diagnostic work has been accomplished for Civil Surgeons and others, from material sent to the Laboratory for that purpose. As it is the intention of Government to make this Laboratory a Provincial Research Institute in addition to its present functions, it is probable that the facilities offered will in future be more largely taken advantage of by medical men throughout the Presidency.

The material received was sent for the following diseases :—

(*a*) Serum diagnosis of Typhoid and Malta Fever	...	38 cases.
(*b*) Examination for Tubercle	...	3 „
(*c*) Examination for Plague	...	2 „
(*d*) Miscellaneous, including tumours, etc.	...	42 „
Total	...	85 cases.

From the local Zoological Gardens a large number of animals were received for post-mortem examination, and opinion as to the cause of death.

Among unusual causes of death may be noted, perforation of the small intestine in a Black Bear from impaction of a mango stone, and impaction of a green cocoanut in the gizzard of a Cassowary, which produced death from inflammation of the stomach and small intestine. A Kangaru was found to have died of the fashionable disease, appendicitis.

The total number of animals examined was 183.

8. *Identification of Mosquitoes.*—A large number of mosquitoes for identification were received from all parts of India, and were dealt with by Captain Liston. No new species were identified out of the 118 received.

9. *Vaccine Lymph.*—Three samples of vaccine lymph received from the Sanitary Commissioner of Bombay and the Inspector-General of Civil Hospitals, Central Provinces, were examined and reported on.

10. *Disinfection and Disinfectants.*—An apparatus for the disinfection by formaldehyde gas, of the boots and shoes of native crews of vessels sailing from Bombay, was further experimented with. The apparatus now in use is the outcome of much labour and experiments by various officers of the Laboratory during the past 4 years. As now modified it appears capable of sterilising boots and shoes in one hour, without injury to the leather. It is now in use by the Health Officer of the Port of Bombay.

Under instructions from the Sanitary Commissioner with the Government of India experiments were undertaken to test the germicidal value of "Chinosol Tabloids" and "Cyllin Special Fluid" with reference to the plague bacillus. Reports on these matters were duly submitted to the Government of India.

11. I take this opportunity of thanking the staff of the Laboratory for the smooth working of the Institute, and their readiness to help by remaining beyond office hours when press of work demanded it.

W. B. BANNERMAN, M.D., Lieut.-Col., I. M. S.,
Director, Plague Research Laboratory.

APPENDIX A.

The following communiqué was issued by the Home Department of the Government of India, Calcutta, December 13th, 1904:—

"At the Health Exhibition organised by the Bombay Sanitary Association under the authourity of the Local Government and in connection with the Industrial and Agricultural Exhibition in Bombay, a series of demonstrations will be given by the Director of the Plague Research Laboratory of the method by which the fluid used for inoculation against plague is now being manufactured. The primary object of these demonstrations is to enable the public to ascertain by personal observation and by enquiry from the experts in charge of the manufacture, the precise nature of the precautions which are now taken to gaurd against the contamination of the fluid by disease-bearing germs. The Director has been instructed to give full explanations of the process to any one who is interested in the subject. The system of inoculation against plague with a fluid prepared from the sterilised virus of disease was introduced in India by Mr. Haffkine early in 1897. The composition of this fluid was subjected to searching enquiry by the Indian Plague Commission, who pronounced its employment to be free from danger, and it was used on a large scale in various parts of India without producing injurious effects. In September 1902 the standard method of manufacturing this fluid was changed by the Director of the Plague Institute, on his own authority, with the object of expediting the process and thus meeting the heavy demand then being made by the Punjab Government in connection with a large scheme of inoculation. The change involved the omission of a small proportion of carbolic acid which had up till then been added to the original fluid as a further precaution against contamination. The new fluid, or water-agar process, contained no carbolic acid, other methods being relied upon to ensure its purity.

On November 6th, 1902, nineteen persons who had been inoculated on October 30th in the village of Malkowal from a single bottle (labelled 53 N) of the new fluid were found to be suffering from tetanus and all of them subsequently died. A Commission consisting of Sir Lawrence Jenkins, Chief Justice of Bombay, Lieutenant-Colonel Bomford, Principal of the Medical College, Calcutta, and Major Semple, Director of the Pasteur Institute, Kasauli, was appointed by the Government of India to enquire into the disaster They found that the germ of tetanus had been introduced into the fluid before the b.ttle was opened at Malkowal, and they thought it probable that this might have occurrod owing either to insufficient sterilisation, or to the process of filling the bottle from a laeger flask having been performed with defective precautions. They also expressed an oprnion that carbolic acid was a valuable agent in restraining tetanus growth when added to tihe plague prophylactic and they therefore thought that its omission was a grave mistake.

Experiments undertaken in India by two independent enquirers appeard to confirm this view, and their conclusions, together with the data on which they were based, were submitted with the report of the Commission for examination and further experiment to the Lister Institute in London. With reference to the findings of the Malkowal Commission, the Institute was asked to report:—(1) On the comparative efficacy of the standard and new fluids as a protection against plague; (2) on the comparative liability of each fluid to contamination; and (3) on the probable origin of the tetanus virus in the Malkowal cases. Their report on these points was received by the last mail. They summarise their conclusions as follows:—(1) The Institute sees no reason to differ from the conclusion of the Commission that the new prophylactic is not less efficacious than the old; (2) the Institute is of opinion that in the hands of more or less unskilled workers it is easier to ensure freedom from contamination by Mr. Haffkine's standard method of manufacturing plague vaccine than with the water-agar process, as employed by him; (3) the Institute is in entire agreement with the Commission as to the value of 0·5 per cent. of carbolic acid in restraining tetanus growth, when added to plague prophylactic, and its experiments emphasize still further the importance of this addition, in preventing growth and toxin formation in vaccine, which might be liable to the possibility of contamination with spores of tetanus; (4) the conclusions of the Institute coincide with those of the Commission that in all probability tetanus was at the time of inoculation in the fluid contained in the bottle, but that it is impossible to determine at what stage in its history, or in what way, the bottle became contaminated.

Some months ago Government decided, on the advice of the Director, that only the standard fluid should be manufactured at the plague institute. The fluid is sterilised by methods approved by the Indian Plague Commission and contains the requisite proportion of carbolic acid. It is now bottled by a new method recently patented by Dr. E. Maynard who was brought out by the Government of India last year for the purpose of perfecting his invention on the spot. It is claimed for this system that it renders it impossible for any germ to enter the fluid from outside, as its efficacy depends, not upon the chemical or bacteriological considerations, but upon a mechanical device, the working of which will be publicly demonstrated at the Exhibition."

APPENDIX B.

Note on some modifications in the method of preparing the medium which serves as a basis in the manufacture of the Plague Prophylactic by Mr. F. Maitland Gibson, M.B., B.Sc. (Edin.)

The fact that albuminous bodies can be hydrolysed, and rendered incoagulable by heat, by the prolonged action of dilute acids or alkalies, or even by plain water, has long been known, and extensively used in the arts.

Such a process, of chemical digestion as it may be called, was first introduced in this Laboratory by the late Lieut-Colonel Warden, I. M. S., in 1898, with the view of avoiding misunderstanding which might have arisen from the use of commercial peptone.

Colonel Warden adopted a system of digestion under steam pressure, with dilute hydrochloric acid. The materials were mixed in the following proportions:—

Cleaned and minced goat's flesh	1	kilogram.
Water	2	litres.
Hydrochloric acid	150	c. c.

and were then digested at 3 atmospheres pressure for a period of six hours.

A very perfect solution of the meat was thus obtained, and the filtered solution after being neutralised was diluted by decoction of goat's flesh, in the proportion of one part of the former to six of the latter.

The medium so prepared had a very high colour, and was very variable in composition.

The first attempt at improvement consisted in decolourising the medium with charcoal. This was best attained by stirring up the acid solution with the charcoal, and straining, the subsequent neutralisation throwing down a copious precipitate of phosphates, derived mainly from the charcoal, which carried farther colouring matter down with it.

This led to a great improvement in the appearance of the medium, but it still remained unreliable in composition, as evidenced by the variable way in which plague bacilli grew in it.

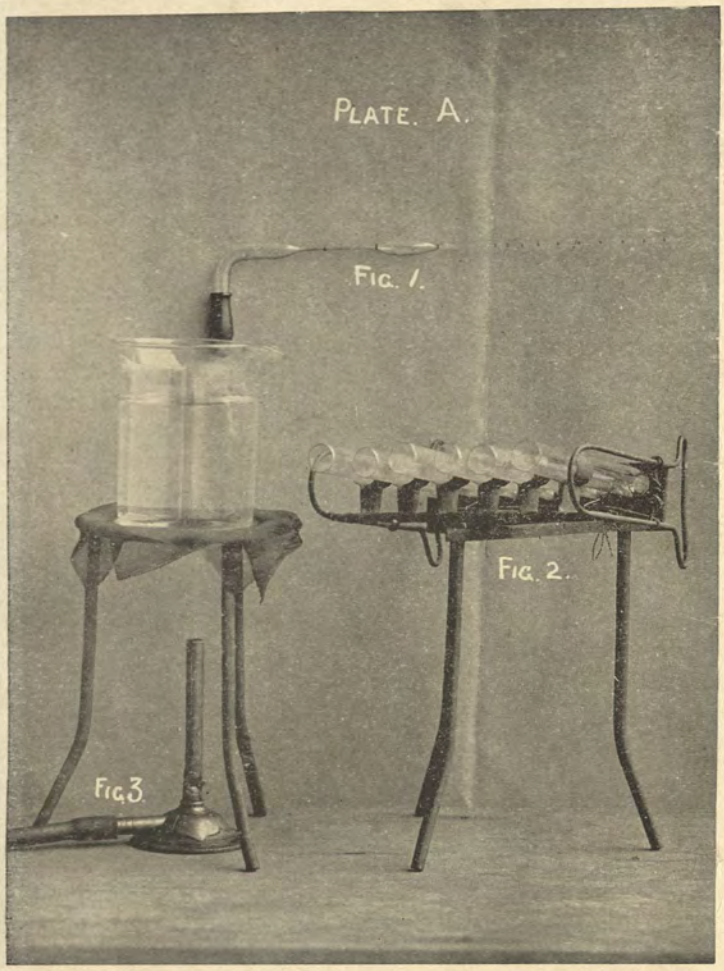
With the idea of avoiding the great dilution necessary to bring the salt, produced on neutralising the hydrochloric acid, down to 0·5 per cent., the quantity of acid was reduced from 150 c.c. to 80 c.c., the proportion of the other materials and the time of heating being the same. The resulting product was then diluted with twice its volume of water. A fairly good medium was thus procured, but it was still not reliable, and the next change tried was that of reducing the temperature of digestion. In all the previous attempts too much stress had probably been laid on getting as perfect solution of the meat as possible, and the likelihood of the more nutritious constituents of the meat being carried beyond the stage of albumose or peptone was not realised. That such did really happen was evidenced by the fact that on warming the finished medium with a little caustic alkali, a strong smell of ammonia and methylamine bodies was evolved. As is well known also, in the Kjeldahl process of nitrogen estimation, the use of concentrated acid and prolonged heating results in the nitrogenous bodies being converted into ammonium sulphate. Considerations such as these led to the trial of a method whereby the heating at high temperatures could be reduced. Maceration at a low temperature was substituted for a part of the heating under pressure and for some years the medium was prepared as follows:

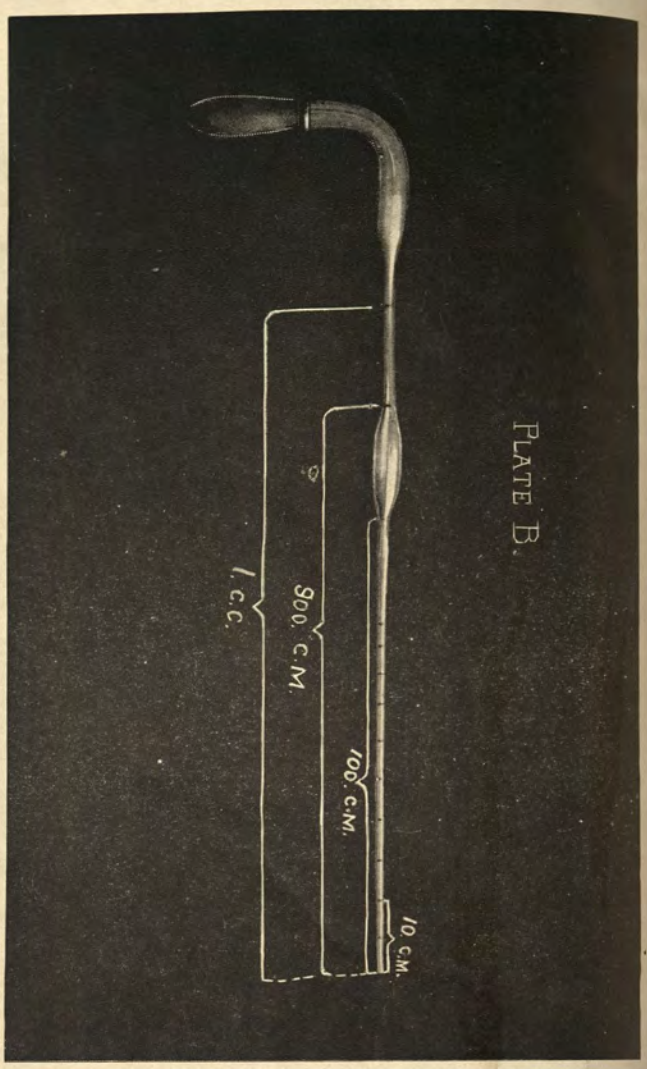
One kilo of cleaned minced goat's flesh was mixed with 80 c.c. of hydrochloric acid, and macerated for a week at a temperature of 70° C. The resulting emulsion was then diluted up to 3 litres and heated at 3 atmospheres pressure for three hours instead of six. The subsequent processes remained as before. In July 1904, experiments were carried out to ascertain the effect of the autoclaving subsequent to maceration.

The meat was mixed and macerated for varying periods. The resulting emulsion being divided into two, one part was autoclaved, and the other simply neutralised, diluted, boiled and strained. The second process produced a much lighter coloured broth which it was unnecessary to decolourise with charcoal. Portions of the finished media thus prepared were evaporated to dryness and the solids weighed. After deducting for the amount of ash (mainly salt, which had been estimated in a separate portion), the following results were obtained:—

Macerated at 70° C.	Not autoclaved.	Autoclaved.
3 days	1·5 per cent.	1·6 per cent.
10 „	1·9 „	2·0 „
17 „	2·71 „	2·76 „
24 „	2·87 „	2·95 „

From these results it will be seen that practically no increase in the total solids was obtained by autoclaving subsequent to maceration. No appreciable difference could be noted qualitatively to the action of ammonium sulphate for albumose, and to copper sulphate for peptone, after eliminating the albumose. Parallel cultivation experiments were next carried out with the autoclaved and simply macerated meat broths. If anything, the advantage seemed to lie with the medium which had not been autoclaved. It seemed





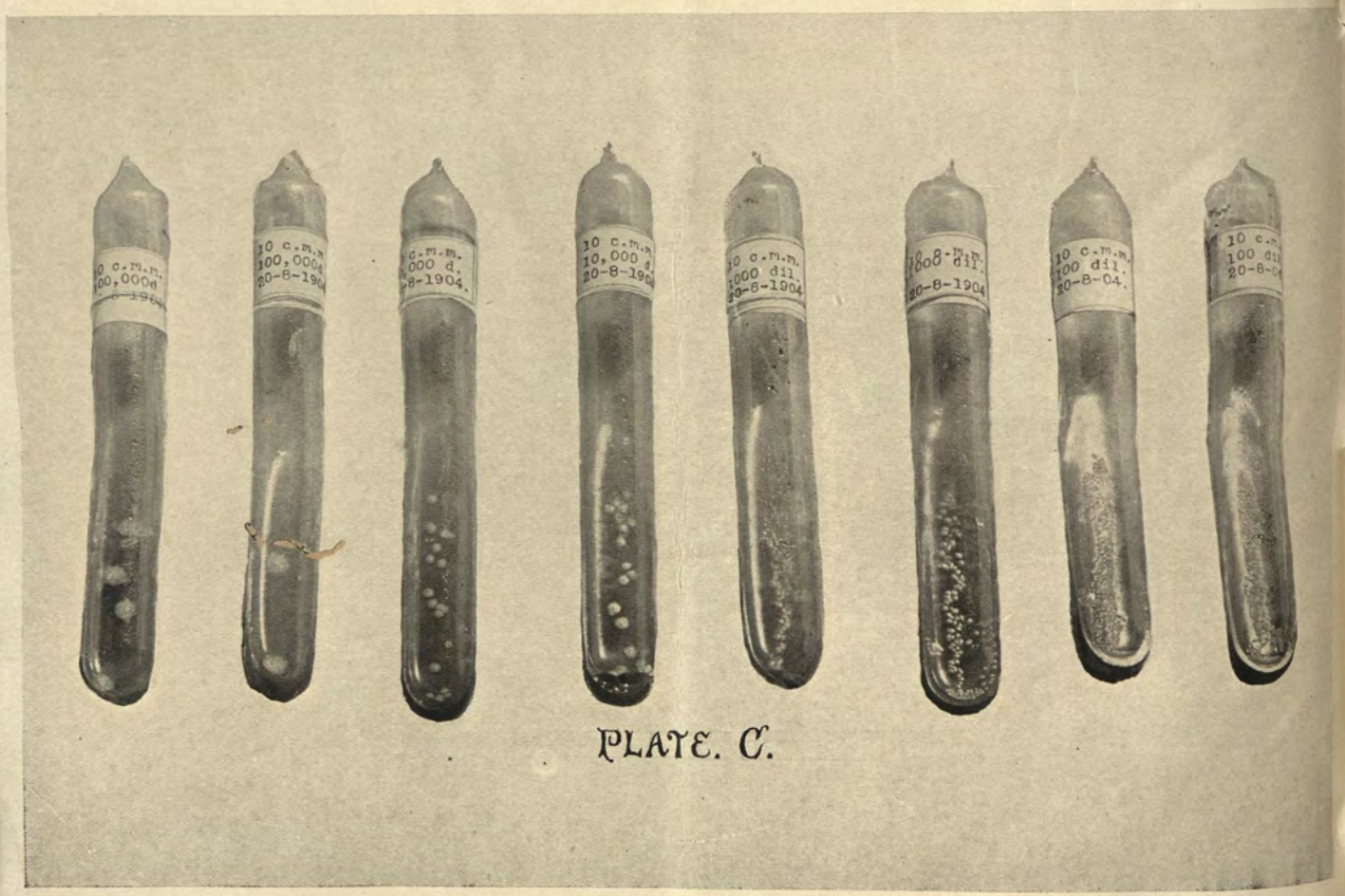


PLATE. C.

rational therefore to do away with the heating under steam pressure altogether, and this carried with it the necessity for decolourising with charcoal. The next thing to be determined was the period of time during which it was desirable to carry on maceration at 70° C. From the figures noted above it will be seen that there is a steady increase of total solids with the length of the period of maceration. This result was largely due to evaporation as it was noticed that the emulsions after maceration were definitely thicker the longer the process had been continued. Further, granting that the more nutritious constituents of the meat are likely to be hydrolysed first, and to undergo splitting up processes by farther heating, it seems desirable to stop short of converting the entire albumen into albumose and peptone. In the broth macerated for three days, about 75 per cent. of the presumably available albumen is converted into albumose and peptone. Parallel cultivation experiments have now been carried out with macerations of three and seven days respectively.

On examination of the comparative cultures after two and four days, it was obvious that the macerations of 3 and 7 days were giving better growths than the autoclaved material. Farther it appeared likely that the maceration of three days was doing better than the seven days one. These results were obtained when using small flasks containing about 100 c.c. of medium each. The relative quality of growth was maintained for six weeks, and there seems no likelihood that it will undergo a change simply on changing the bulk of the fluid. Cultivations on the ordinary working scale are on hand.

N.B.—For those persons who may have objections to the use of a medium made from animal flesh, a bouillon from wheat flour can be prepared on the same principles.

APPENDIX C.

Method used for finding the minimum lethal dose of the plague cultures used.

The apparatus used (*vide* Plate A) consists of:—

(1) A graduated pipette. (1) Plate A Fig. 1, and Plate B.

(2) A stand for holding test tubes. (2)

(3) A beaker with boiling water for washing out the pipette. (3)

The pipette is made from ordinary glass tubing and is graduated with mercury as illustrated.

It will be seen that the total capacity of the pipette is 1 c. c. and that it is possible with ease to make various dilutions.

The stand Fig. 2, Plate A is adapted to hold test tubes on a slight slant.

If 1 c. c. of various dilutions of a plague culture are required, the following procedure is adopted:—

The pipette is first thoroughly sterilised by passing it through the flame of a bunsen several times; it is then allowed to cool. Meanwhile sterile test tubes are adjusted in the stand. The cotton wool plugs are removed from these and the mouths of the tubes sterilised by the bunsen flame.

The pipette is then filled with 1 c. c. of sterile broth. The pipette is introduced into the first test tube and its contents emptied save for 100 cubic millimetres. A series of six or eight test tubes are in this manner filled with 900 cubic millimetres of sterile broth.

A 48 hours culture of plague in rat broth is next taken in hand and from this 100 cubic millimetres are extracted by the pipette and introduced into the first tube containing 900 millimetres of broth. The broth and culture are drawn two or three times into the bulb of the pipette by squeezing and relaxing the teat affixed to the pipette, a thorough mixture is thus effected. The contents of the pipette are then completely ejected into the first tube which now contains a 1 in 10-fold dilution of the original broth plague culture. Boiling water is next drawn in and out of the pipette so that it is thoroughly cleaned and sterilised. The pipette is then passed through the flame to dry it and it is allowed to cool. One hundred cubic millimetres of the first tube is now transferred to the second tube and the mixture of the 1 in 10-fold dilution of the culture and sterile broth is effected as described above, so that in the second tube a 1 in 100-fold dilution of the original culture is obtained. The third tube is treated in the same way with 100 c.m.m. of the 1 in 100-fold dilution so that it contains a 1 in 1,000-fold dilution of the original culture. Other tubes are similarly prepared till a 1 in 10 million-fold dilution is obtained. In $\frac{1}{4}$ of a c. c. of this dilution not more than one or two plague germs are ever present if a 48 hours' rat broth culture is used.

Care has to be taken (1) to allow sufficient time for the drainage of the pipette each time the sterile broth is introduced into the sterile test-tubes. This is best accomplished if the pipette is almost completely emptied and broth again drawn up to the mark indicating 100 cubic millimetres measure. (2) Great care must be taken to completely sterilise and clean out with boiling water the pipette between the preparation of each dilution. (3) The plague germ is so readily killed by heat (a temperature of 50° C. greatly injures the germ even in a few minutes and completely destroys it in 15 minutes) that great care has to be taken that the pipette is quite cool before using it after sterilisation. The bulb which is of thick glass retains the heat longest, it should always be felt to be quite cool after sterilisation and before introducing the living germs into it.

When the dilutions are completed 30 cubic millimetres are introduced into the pipette from the million-fold dilution. Ten cubic millimetres of this are spread out on the surface of a sloped agar tube by gently squeezing the pipette and smearing the liquid thus extruded by means of the stem of the pipette on the surface of the agar. Three tubes are thus treated. Three similar tubes are prepared from the one-hundred-thousand-fold dilution and three from the ten-thousand-fold dilution.

These tubes are allowed to incubate and after three days the colonies that have developed in each tube are counted and from the figures so obtained an estimate can be made of the number of living germs present in each c. c. of the original culture.

Plate C shows cultures containing colonies planted out in the above manner.

The dilutions employed in this case being 1-100; 1-1,100; 1-10,000 and 1-100,000.

APPENDIX D.

Anti-Plague Inoculation at Aden.

The plague epidemic started in the Crater about the end of October 1904, reached its maximum intensity between December 10th, 1904, and February 26th, 1905, rapidly declined during March and stopped at the end of April. 9,374 inoculations were done between December 16th, 1904, and April 20th, 1905. 2,571 inoculations were done in December 1904, 3,895 in January 1905, 1,797 in February, 1,086 in March, and 25 in April. The fact that the Crater possessed a large homeless and migrating population, made the compilation of accurate statistics for the whole of the town quite impossible, especially as nearly all the inoculations were done amongst these homeless people.

The Jewish community was picked out as the most likely to afford the nearest approach to accuracy, especially as Mr. Menahim Messa, the leading member of the community, together with some volunteers amongst whom Mr. Howard, the Manager of the Aden Press, was prominent, had kept control of their people throughout the epidemic and had persuaded many to be inoculated and to carry out other measures of protection, and were willing to give every particular of plague cases and inoculations amongst them.

Accordingly a house-to-house inspection was made of the houses in which plague cases had occurred, and the result is given in the tables that follow:—

Jewish Community in the Crater.

Uninoculated.	Cases.	Deaths.	Inoculated.	Cases.	Deaths.
149	45	24	75	10	2

Of the 149 uninoculated Jews, 58 were inoculated after a plague case had occurred in their houses; there were no plague cases amongst these 58 people. Evacuation of the house was carried out after a plague case had occurred, so that the above table represents the state of affairs before evacuation was carried out.

The following table shows the percentage result in each group:—

Uninoculated.	Cases per cent.	Deaths per cent.	Cases Mortality per cent.	Inoculated.	Cases. per cent.	Deaths per cent.	Cases Mortality per cent.
149	30·2	16·1	53·3	75	13·3	2·6	20·0

If the inoculated had suffered in the same proportion as the uninoculated, they should have had 12 deaths instead of 2; a difference of 83·3 per cent.

Native Town, Crater.—A house-to-house inspection was also made of the infected houses in the rest of the Native Town, but very few inoculated people were found in them, partly no doubt because many had left Aden, and their houses were found to be occupied by newcomers. The table below shows the results:—

Uninoculated.	Plague		Inoculated.	Plague	
	Cases.	Deaths.		Cases.	Deaths.
734	285	243	92	16	8

Of the 734 uninoculated inhabitants, 112 were inoculated after a plague case had occurred in their houses; there were no cases of plague amongst these 112 people.

Uninoculated.	Cases per cent.	Deaths per cent.	Case Mortality per cent.	Inoculated.	Cases per cent.	Deaths per cent.	Case Mortality per cent.
734	38·8	33·1	85·2	92	17·3	8·7	50·0

If the inoculated had suffered in the same proportion as the uninoculated, they should have had 30 deaths instead of 8; a difference of 73·3 per cent.

Special Prison, Crater.—At the Special Prison two cases of plague occurred, in one case the man was supposed to have been inoculated at Sheik Othman, and accordingly was not done on admission, in the other the man was in the prison for some days before being inoculated, and he probably was incubating plague when operated on, as there was no interval between the inoculation fever and the plague fever. It may be mentioned that dead rats continued to be found in the prison during the epidemic. The table below shows the results:—

Probably uninoculated at time of attack.	Cases.	Deaths.	Inoculated.	Cases.	Deaths.
2	2	1	85	0	0

Native Infantry; Sepoys and Followers, etc., in Crater.—The Native Infantry who were inoculated soon after the first plague case, show very good results; they however also moved into camp on the parade ground close by the lines.

The following table gives the results:—

Uninoculated.	Cases.	Deaths.	Inoculated.	Cases.	Deaths.
94	7	5	405	3	1

Uninoculated.	Cases per cent.	Deaths per cent.	Case Mortality per cent.	Inoculated.	Cases per cent.	Deaths per cent.	Case Mortality per cent.
94	7·4	5·3	71·4	405	0·7	0·2	33·3

If the inoculated had suffered in the same proportion as the uninoculated, they should have had 21 deaths instead of 1 death; a difference of 95·2 per cent.

Maala.—Extremely few of the inhabitants of Maala were inoculated, the 826 inoculations done there being amongst the homeless and the coolies who worked at the wharves. The result is shown in the following table:—

Census of plague-infected houses containing both Inoculated and Uninoculated people.

Uninoculated.	Cases.	Deaths.	Inoculated.	Cases.	Deaths.
6	2	2	5	0	0

Digitized with financial assistance from the Government of Maharashtra on 11 July, 2017

Of the 6 uninoculated inhabitants, 4 were inoculated after a plague case had occurred in their houses; there were no cases amongst these 4 people.

Tawahi.—At Tawahi also very few inoculated people were found in the infected houses, in this case partly because many of them had probably left Aden, as many infected houses were found to be occupied by newcomers.

Census of plague-infected houses containing both Inoculated and Uninoculated people.

Uninoculated.	Cases.	Deaths.	Inoculated.	Cases.	Deaths.
58	20	14	16	2	1

Of the 58 uninoculated people, 29 were inoculated after a plague case had occurred in their houses, none of these were attacked by plague.

The following shows the percentage result:—

Uninoculated.	Cases per cent.	Deaths per cent.	Case Mortality per cent.	Inoculated.	Cases per cent.	Deaths per cent.	Case Mortality per cent.
58	34·5	24·1	70·0	16	12·5	6·2	50·0

If the inoculated had suffered in the same proportion as the uninoculated, they should have had 4 deaths instead of 1; a difference of 75·0 per cent.

Aden Troop Sowars and Followers at Khor-maksar.—A plague case occurred in the Lines on the 5th, and another on the 10th of February 1905; inoculations were commenced on the 12th and the majority finished by the 18th of February.

The following table shows the results :—

Uninoculated.	Plague		Inoculated.	Plauge	
	Cases.	Deaths.		Cases.	Deaths.
9	7	5	220	6	2

The following shows the percentage result in each group:—

Uninoculated.	Cases per cent.	Deaths per cent.	Case Mortality per cent.	Inoculated.	Cases per cent.	Deaths. per cent.	Case Mortality per cent.
9	77·7	55·5	71·4	220	2·7	0·9	33·0

If the inoculated had suffered in the same proportion as the uninoculated, they should have had 122 deaths instead of 2; a difference of 98·3 per cent.

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Bombay, 18th August 1905.

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